TEMPORAL VARIATIONS IN HISTOLOGICAL APPEARANCE OF THYROID AND PITUITARY OF SALAMANDERS TREATED WITH THYROID INHIBITORS[1]

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The fact that thiourea and a number of related chemical compounds are capable of interfering in some way with the normal activity of the vertebrate thyroid gland is now well known. Administration of these substances to common laboratory mammals causes an almost immediate decrease in the thyroid hormone level as evidenced by a lowered rate of metabolism and other symptoms of hypothyroidism. With continued treatment the thyroid gland becomes hypertrophic and hyperplastic, presumably because the decreased content of thyroid hormone in the blood induces a compensatory over-production of thyrotrophic hormone by the pituitary gland. The thyroids of treated animals may thus become markedly enlarged and these thyroid-inhibiting drugs are therefore often referred to as goitrogens.

An extensive literature is available concerning the effects of various goitrogens upon the morphology and physiology of the thyroid gland and the possible mechanism of action of the drugs in laboratory mammals and in the human subject. (See reviews of Charipper and Gordon, 1947; Trotter, 1949; Astwood, 1949; Pitt-Rivers, 1950; Lever, 1951.) Relatively little work has been done on the effects of these compounds on lower vertebrates. However, there is sufficient information available to indicate that they are effective to greater or lesser degree in all types of vertebrates and probably act in essentially the same way in all (Lynn and Wachowski, 1951). Among amphibians the effectiveness of thiourea and related compounds is readily shown by their ability to inhibit metamorphosis in the tadpole (Gordon, Goldsmith and Charipper, 1943; Hughes and Astwood, 1944; Lynn and De Marie, 1946; Koch, 1948; Harms, 1949; Delsol, 1948). However, study of the thyroid glands of treated tadpoles has revealed that although the histological changes observed are similar to those reported for mammals, they are less marked, and after long-continued treatment a decrease in thyroid size and activity may be observed (Gordon, Goldsmith and Charipper, 1945). This decrease also occurs in the adult frog, and Joel, D'Angelo and Charipper (1949) found that after such "regression" the gland may be reactivated by administration of thyrotrophic hormone. It thus appears that the indications of decreased activity observed in the thyroid after long-continued treatment with goitrogens are due to the exhaustion or the impairment of the pituitary thyrotrophic function. Adams (1946) reported that adult salamanders (*Triturus viridescens*) exhibit relatively slight thyroid hyper-

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plasia after long-continued treatment with thiourea. It has been suggested (Lynn and Wachowski, 1951) that this may also be due to a "regression" similar to that noted in the frog.

The present investigation is a study of the histology of both the thyroid and pituitary glands in salamanders treated for periods varying from 5 to 90 days with several different concentrations of four different goitrogens. This study was undertaken in an attempt to ascertain more precisely the effects of these various treatments upon the thyroid, and to find out whether there are any histological changes in the pituitary which can be correlated with the thyroid response.

Materials and Methods

The salamanders used in the present experiments were adult specimens of *Desmognathus fuscus brimleyorum* Stejneger obtained from Dr. Charles Burt of Topeka, Kansas. The animals were kept under laboratory conditions for about a month before use. During this time they exhibited normal activity and a very low mortality rate.

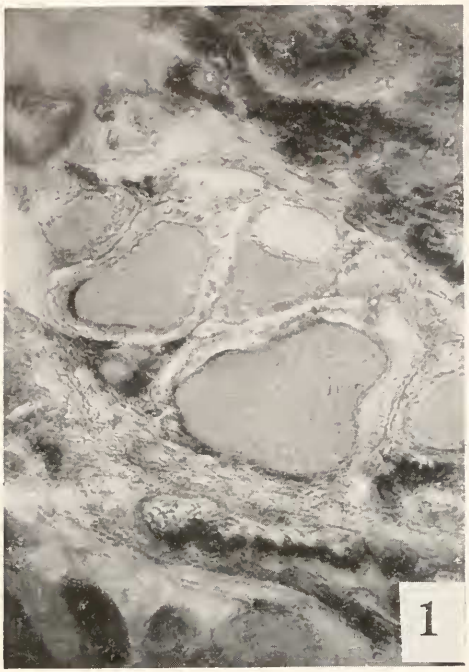
Treatment with thyroid-inhibitors was carried out simply by maintaining the specimens in culture solutions consisting of the appropriate concentrations of the drugs in tap-water. The animals were kept in large finger bowls containing one liter of solution, with seven or eight animals to a bowl. The solutions were changed weekly. The salamanders were fed earth-worms about once a month. At regular intervals, control and experimental animals were removed from the solutions and anesthetized with MS 222. From these animals thyroids and pituitaries were removed. The thyroid glands were fixed in Bouin's fluid and the pituitaries in Zenker's fluid. After two hours fixation, which proved to be sufficient, the material was washed in running water, dehydrated in Cellusolve, cleared in oil of wintergreen and embedded in paraffin. Thyroids were sectioned at 8 μ and stained with Mallory's triple stain. Pituitaries were sectioned at 5 μ and stained according to the technique of Pearse (1950).

In addition to several sets of controls kept in tap-water, the following concentrations of goitrogens were used: 0.005%, 0.01%, 0.05%, 0.1%, 0.3%, 0.5% and 1% thiourea; 0.005%, and 0.01% phenylthiourea; 0.05% allylthiourea; 0.05% thiouracil.

Results

A. Effect of treatment with goitrogenic agents upon the histology of the thyroid gland

All of the treatments used in the present study were effective in producing well-defined histological changes in the thyroid gland of the salamander. However, as one would expect, the effects observed and the time required for their appearance varied with the different drugs and with different concentrations of the same drug. These effects are similar to those recorded in the literature for other vertebrates such as the rabbit (Baumann, Metzger and Marine, 1944) and the chick (Mixner, Reineke and Turner, 1944) and are in particularly close agreement with those reported for other amphibia (Gordon, Goldsmith and Charipper, 1943; Adams, 1946; Gasche and Druey, 1946; Blakstad, 1949; Lynn, 1948; Joel, D'Angelo and Charipper, 1949).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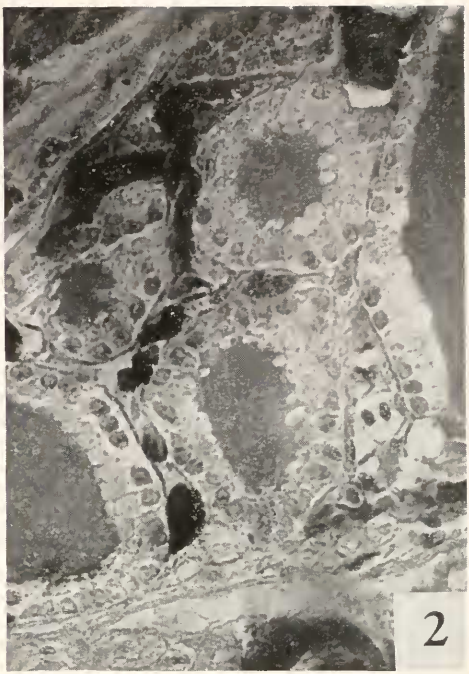
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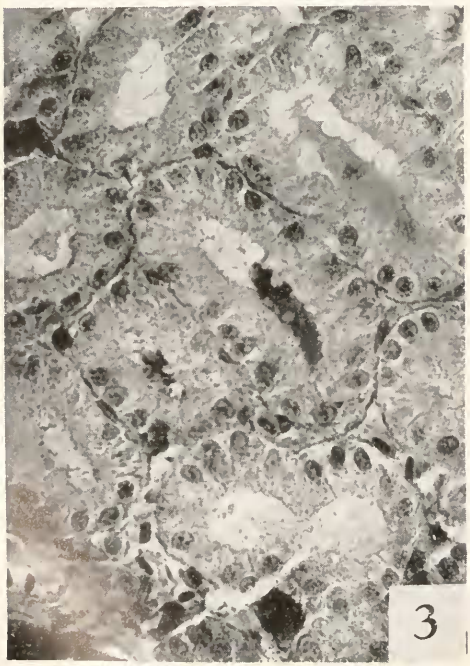
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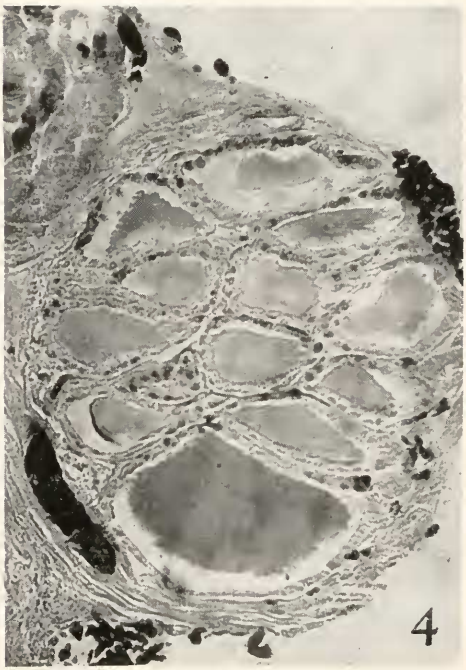
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The general sequence of changes observed in the thyroid following treatment with a goitrogen may be briefly outlined as follows. The first indication of an effect is usually seen in an increased vascularity of the gland. This is often grossly discernible in the intact thyroid and is readily detectable in sectioned material where the hyperemia is indicated by enlarged vessels and an increased number of blood cells (Fig. 2). This change is usually followed in a short time by the appearance of chromophobe droplets ("vacuoles") within the epithelial cells and around the periphery of the colloid mass (Fig. 2). These intracellular and intrafollicular vacuoles are generally considered as reliable indications of increased activity in the gland and they continue to increase in size and number with time. As the vacuolization progresses, the form of the follicular epithelium gradually changes from flat or cuboidal to high columnar and at the same time the staining reaction of the intrafollicular colloid undergoes an alteration from acidophilic to basophilic. Following this the amount of intrafollicular colloid may be greatly decreased so that many follicles have their lumina completely obliterated (Fig. 3), the walls of the follicles being extremely thick and often much folded. Hyperplasia may occur, as indicated by an increase in the number of mitotic figures observed in the epithelium and the whole gland may become grossly hypertrophied. However, such hypertrophy was never so marked as that which is produced by comparable treatment in mammals or even in larval amphibians. After long-continued treatment with relatively high concentrations of goitrogens the appearance of the tissue may return almost to normal with decrease in the height of the epithelium and renewed storage of acidophilic intrafollicular colloid (Fig. 4). The basis for this "regression" will be discussed at length later.

It will be remembered that in these experiments a rather wide range of concentrations of thiourea was investigated while only a few concentrations of three other goitrogens were employed. Using as a basis the foregoing general account of the effects of these substances upon the thyroid, the detailed results of each of the experimental treatments will now be considered.

1. 0.005% thiourea

Thyroids of animals raised in this concentration of thiourea showed no differences from control glands (Fig. 1), until 15 days after the beginning of treatment. At this time there was a definite increase in vascularity and a few intracellular and intrafollicular vacuoles were apparent in some but not all follicles of the experimental animals. By the 25th day of treatment the staining reaction of the colloid mass was basophilic but the follicular epithelium was still cuboidal and the number of vacuoles had not significantly increased. Specimens killed at later periods up to the 88th day of treatment, when the experiment was terminated, exhibited essentially this same condition except for a slight increase in the number of intrafollicular

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FIGURE 1. Photomicrograph of normal thyroid gland in *Desmognathus fuscus brimleyorum*. × 150.

FIGURE 2. Photomicrograph of a thyroid gland from an animal treated for 25 days with 0.05% thiouracil. × 280.

FIGURE 3. Photomicrograph of a thyroid gland from an animal treated for 25 days with 0.05% thiourea. × 280.

FIGURE 4. Photomicrograph of a thyroid gland from an animal treated for 85 days with 0.05% thiouracil. × 150.

vacuoles. In none of the specimens studied did the epithelium become columnar nor was there any indication of a decrease in colloid volume.

2. 0.01% thiourea

Clear-cut effects appeared in this series within 10 days after the beginning of treatment. At this time there was marked hyperemia, the follicular epithelium had become low-columnar, both intrafollicular and intracellular vacuoles were abundant and in most follicles the colloid mass, while still acidophilic at the center, showed a wide peripheral zone of basophilic material. These features persisted in the later specimens but the amount of colloid in the gland gradually decreased so that by the 37th day of treatment many follicles appeared to be completely empty. By 72 days the epithelium had become extremely high and the colloid content of the gland was even further diminished. This condition still existed on the 90th day when the experiment ended (Fig. 3).

3. 0.05% thiourea

The effects of this treatment corresponded with those obtained with 0.01% thiourea during the early phases of the experiment. At 10 days the epithelial height was only moderately increased but vacuolization, increased vascularity and basophilia in the colloid were clearly evident. The epithelium became tall columnar by the 15th day, however, and by the 25th day the amount of stored colloid in the gland had been markedly reduced (Fig. 3). This represents the maximum effect obtained with this concentration and the histological condition presented by the thyroid remained unchanged throughout the remainder of the experimental period (72 days).

4. 0.1% thiourea

Specimens exposed to this concentration for 10 days showed striking effects which included not only hyperemia and increased vacuolization but also hypertrophy of the epithelium and decrease in colloid volume. The latter had already proceeded to such a degree that many follicles were completely collapsed. This condition persisted through the 54th day of treatment but in the thyroids of animals killed at later periods there were some indications of a decrease in activity of the thyroid. These indications consisted in some slight reduction in epithelial height and a gradual but rather marked increase in the stored colloid volume. In the specimens tested longest (85 days) some acidophilic colloid was present.

5. 0.3% thiourea

As in the immediately preceding series the earliest specimen studied (10 days) already showed what might be considered almost maximal effects. Material treated for longer periods also corresponded with that seen in the specimens treated with 0.1% thiourea so that this series shows no significant difference in the effectiveness of these two concentrations.

6. 0.5% thiourea

Although animals treated with 0.1% and 0.3% showed marked histological changes in the thyroid within 10 days, specimens treated with 0.5% thiourea did not show such effects. Even up to 25 days of treatment the thyroids of these animals differed from those of the controls only in displaying a slight hyperemia, low columnar epithelium and a moderate degree of intrafollicular vacuolization. By the 37th day the vacuolization had greatly increased and some follicles had a depleted colloid content. However, this seems to have been the period of maxi-

mum effect for in specimens examined after the 37th day the colloid content was gradually increased, the epithelium decreased in height and by the 72nd day of treatment the gland had almost regained the appearance seen in the controls.

7. 1.0% thiourea

The effects of this, the highest concentration of thiourea used, were similar to those obtained with 0.5% thiourea. No significant changes occurred until the 25th day. These increased to a maximum at about the 54th day and then there was a well-defined "regression." However, the decrease in activity had not proceeded so far as to produce a gland of normal appearance when the experiment was terminated at 90 days.

8. 0.005% phenylthiourea

This concentration of phenylthiourea proved more effective than the same concentration of thiourea. Animals treated for 10 days exhibited numerous intracellular and intrafollicular vacuoles and marked hyperemia of the gland. By the 15th day the epithelium had become extremely high and depletion of the colloid content was already far advanced. This same condition was presented by the glands of animals treated up to 54 days but following this a well-defined decrease in activity occurred which was more striking than that seen in any of the thiourea treatments. The height of the epithelium gradually decreased until by 72 days all follicles were lined by flattened cells. Intracellular and intrafollicular vacuoles entirely disappeared and the colloid became dense, homogeneous and completely acidophilic. The gland of the experimental animal at this time thus gave the appearance of a much more inactive thyroid than is seen in the average control.

9. 0.01% phenylthiourea

The effects with this concentration were even more striking than those obtained with 0.005% phenylthiourea. After only 10 days treatment much of the stored colloid had disappeared and by the 25th day there was almost no colloid present in any of the follicles. Specimens given longer treatment show evidence of "regression" although this is not so marked as in the immediately preceding series.

10. 0.05% phenylthiourea

This concentration represents the maximum solubility for this drug and it proved toxic to these animals in a short period. The thyroid of one specimen which survived for 5 days in this concentration was available for study. It is of interest since it already showed increased epithelium height and extreme vacuolization and thus furnished some indication that this concentration is probably even more effective than the preceding.

11. 0.05% thiouracil

This treatment gave no significant results until the 25th day. At this time the epithelium height had increased, intrafollicular vacuoles were abundant and the gland had become hyperemic (Fig. 2). The effects became more pronounced at later stages but by the 85th day there was clear evidence of decreased activity, the epithelium having become low columnar to cuboidal and the colloid having increased in amount (Fig. 4).

12. 0.05% allylthiourea

The effect of this treatment was not apparent until the 37th day but by the 54th day quite marked effects were obtained, the colloid being completely exhausted and the whole gland hypertropic. As in the case of treatment with 0.05% thiouracil,

this was later followed by decreased activity, the epithelium becoming low and the colloid abundant in the later stages studied.

B. Effects of treatment with goitrogenic agents upon the histology of the pituitary

The fact that the thyroid gland is activated by a thyrotrophic principle had well been demonstrated in a number of ways. But precisely which cells of the pituitary are responsible for the elaboration of this hormone is still open to question. One of the aims of the present study was to obtain some evidence on this point.

The generally accepted concept of the mode of action of the goitrogenic substances used in this work is as follows. These drugs interfere in some way with the normal synthesis of the thyroid hormone. As a result of this the thyroid hormone level in the blood is decreased and this decrease stimulates the anterior lobe of the pituitary to produce and/or release its thyroid-activating hormone. The histological changes in the thyroid thus result from stimulation by the thyrotrophic hormone. If the goitrogenic substances are administered continuously, the thyroid, despite its increased cell height and general hypertrophy, continues to be hypofunctional and therefore the pituitary continues to release the thyrotrophic hormone and a goiterous condition results. If a particular cell type in the pituitary is responsible for secretion of the thyrotrophic hormone it would be expected that in animals in which these cells have been over-stimulated by long-continued treatment with goitrogens, some histological changes involving them should be observable. In the material under investigation there is also the possibility that in those animals in which later decreased activity of the thyroid was observed, some histological evidence of the basis for this decrease might be seen in the pituitary. It has been suggested (Joel, D'Angelo and Charipper, 1949) that the thyroid "regression" comes about because the thyrotrophic hormone-secreting cells of the pituitary, after long-continued over-activity, become exhausted and fail to secrete any longer. This would result in a reduction in the thyroid-activating principle in the blood and a consequent return of the thyroid to a normal, or more probably, to an extremely inactive condition.

Although both the pituitary and thyroid glands of all animals were studied, detailed consideration of the histology of the pituitary will be limited to points which bear upon the question of secretory specificity of the cells of the anterior lobe. It is obvious that the most important material to be considered will be pituitaries from animals whose thyroids showed maximum hypertrophic effects and pituitaries from animals which showed the most clear-cut evidence of decreased thyroid activity after long-continued treatment. These, along with pituitaries of control animals, form the basis for the account which follows.

The general appearance of the anterior lobe of the pituitary in the salamander, *Desmognathus fuscus brimleyorum,* with the staining technique employed here is as follows. The acidophilic elements show a clear yellow-staining cytoplasm with a relatively large centrally located nucleus; the basophilic elements exhibit a somewhat excentric nucleus and a cytoplasm which is heavily granulated, and magenta-staining; the chromophobic elements, which are difficult to identify in many cases, show a pale cytoplasmic background and a heavily staining nucleus. The nucleus stains dark blue in all three types of cells.

The first indication of changes in the pituitary were found in animals whose thyroids already exhibited well-marked signs of inhibition. Study of a series of animals in which the thyroids showed progressive activation revealed that the pituitary glands were undergoing concurrent changes characterized by a gradual increase in the proportion of basophilic elements and a decrease in the relative numbers of acidophilic elements. At the same time the chromophobic elements exhibited an increased granulation which may possibly represent transition toward a basophil type.

A study of the pituitaries of specimens whose thyroids exhibited decreased activity after long-continued treatment revealed that the basophilic elements were still predominant but there was evidence of some degenerative change in these cells. The cell outlines tended to be indistinct, the cytoplasm stained less uniformly and many cells had distorted, seemingly pycnotic, nuclei. There was no evidence in this material of any increase in the relative numbers of acidophilic elements. These results tend to support the concept that the decreased activity of the thyroid after long-continued treatment with goitrogens is not due to any return of the pituitary to normality but rather to an abnormality of the basophil cells of the anterior lobe probably resulting from their over-activity and consequent exhaustion.

Discussion

As has been noted, all of the treatments used in the present study produced well-defined histological changes in the thyroid gland but the extent of the changes and the time required for their appearance differed with the treatment. Since thiourea was the only drug for which a wide range of concentrations was tested, conclusions concerning the relation between concentration and goitrogenic effects must be limited to this substance. It will be remembered that treatment with 0.005% thiourea, the lowest concentration tested, was not sufficient to cause maximal activation of the thyroid during the experimental period of approximately three months. With 0.01% thiourea detectable changes in the thyroid appeared sooner and maximal effects were apparent by the 72nd day of treatment. The next higher concentration, 0.05% thiourea, caused an even earlier maximal activation. It appears, however, that the optimal concentration with respect to rapidity of effect lies in the region of 0.1% to 0.3%. These concentrations gave essentially the same results. The thyroids of animals treated for only 10 days appeared to be already fully activated and the condition remained unchanged during the subsequent two months of treatment. During the third month of the experiment the thyroids of animals given these concentrations exhibited signs of a gradual decrease in activity which resulted finally in a histological condition indicating marked inactivity of the gland. The two highest concentrations of thiourea tested, 0.5% and 1.0%, caused less marked changes in the thyroid than did 0.3% or 0.1%. It thus appears that in the present material, the goitrogenic effects of thiourea administered in the culture medium increase with the concentration of the drug up to an optimal concentration at 0.1% to 0.3% and decrease at higher concentrations.

Most of the studies on the effects of thyroid-inhibiting drugs in amphibians have dealt with only one or two concentrations. Bruce and Parkes (1947), however, report that the metamorphosis of larvae of *Discoglossus pictus* is only slightly

delayed when the animals are kept in 0.04% thiourea but is completely inhibited by 0.1% thiourea. Use of 0.2% and 0.5% solutions prevented metamorphosis but also affected the growth of the tadpoles adversely and resulted in a high mortality. Lynn (1948) found that larvae of *Eleutherodactylus ricordii* showed no effects on their growth or metamorphic pattern when treated with 0.001% thiourea, but were increasingly affected by concentrations of 0.005% and 0.05%. The thyroids of the larvae treated with 0.05% thiourea exhibited marked changes but those of animals from the two lower concentrations were not significantly affected. The work of Ratzersdorfer, Gordon and Charipper (1949), although it deals with a lizard rather than an amphibian, is also of interest in this connection for they found that high concentrations of thiourea when administered by injection produced less marked effects on the thyroid than did lower concentrations. These authors ascribed this result to (p. 23) "toxic reactions which tend to mask partially the goitrogenic effect." The nature of the toxic reactions involved is uncertain but it does seem likely that for any goitrogenic drug there is, for any specific animal, an optimal concentration above which less pronounced effects are obtained. This fact may account for the results reported by Adams (1946) who found that the thyroid of adult *Triturus viridescens* which had been kept in thiourea solutions for periods up to 86 days showed relatively slight changes. These newts were in two groups and had been treated with 0.033% and 0.066% thiourea during the first 28 days of the experiment but the concentrations were later increased in two steps so that for the last 44 days the concentrations were 0.528% and 1.056%. In the light of the present results it seems probable that these latter concentrations were above the optimal level and were, therefore, less effective than somewhat lower concentrations would have been.

Of the four different goitrogens tested in the present study, phenylthiourea appears to have the greatest potency. At a concentration of 0.005% this drug produced effects comparable to those obtained with 0.1% thiourea. Thiouracil at a concentration of 0.05% gave effects similar to those resulting from treatment with the same concentration of thiourea while allylthiourea at the same concentration, though its effect was delayed, seemed to produce more marked changes than thiourea. Arrangement of the drugs in decreasing order of effectiveness would thus be: phenylthiourea, allylthiourea, thiourea, thiouracil, with the last two, as far as the present evidence indicates, being approximately equivalent.

Although the relative potencies of a very large number of goitrogens have been tested for common laboratory mammals (Astwood, 1943; Astwood, Bissell and Hughes, 1945) there is very little information available concerning the relative effectiveness of these drugs in cold-blooded animals. Lynn's (1948) results with phenylthiourea and thiourea administered to larvae of the toad *Eleutherodactylus ricordii*, are in agreement with the present results in indicating a greater potency for the former substance. Gasche and Druey (1946) reported that allylthiourea is 10 to 20 times as effective as thiourea in inhibiting metamorphosis in Xenopus larvae and Koch (1948) also found indications of a high potency for allylthiourea in the inhibition of metamorphosis of *Rana temporaria* larvae. Thomas (1947) found that both phenylthiourea and allylthiourea show a greater effectiveness than thiourea when tested on *Rana pipens* tadpoles but he gives no information on the relative potency of the two first-named drugs. Because of differences in the meth-

ods of administration and in the criteria for effectiveness, the results of these studies, so far as they relate to the relative potencies of the goitrogens in question, are difficult to evaluate. It is clear that a detailed assay of the various goitrogens is necessary before any conclusions can be drawn concerning the relative potency of the drugs in amphibians.

The fact that long-continued treatment with thiourea results in decrease in the size and epithelial height of the thyroid was noted in the first investigations of the effects of goitrogen on amphibian material (Gordon, Goldsmith and Charipper, 1943, 1945). These authors found that *Rana pipiens* larvae treated with 0.033% thiourea for three weeks showed slightly enlarged and markedly activated thyroid glands. This condition persisted until about the seventh week of treatment but after this a "regression" occurred which resulted in a decrease in size of the gland as well as a marked change in its histology. Later study by Joel, D'Angelo and Charipper (1949) showed that this same phenomenon occurs in the adult frog after prolonged thiourea treatment and also that the gland can be reactivated by administration of thyrotrophic hormone. These authors, therefore, conclude that the thyroid "regression" is due to a failure in the production or the release mechanism of the thyrotrophic hormone or to a failure in both. It has been seen that in the present experiments a number of the treatments tested, notably those which produced histological evidence of early and maximal thyroid activation, resulted in histological changes indicating decreased thyroid activity after long-continued administration. Thus adult salamanders, like both larval and adult frogs, exhibit this phenomenon.

Since the thyroid activation and the later "regression" are presumed to result from changes in the level of thyrotrophic hormone produced by the pituitary gland, the pituitaries of the experimental animals were subjected to study. It was found that the pituitaries of animals whose thyroids showed well-defined signs of activation differed from those of normal animals in showing an increase in relative numbers of basophilic cells and a decrease in acidophils. In specimens whose thyroids were undergoing "regression" basophils were still predominant but there was evidence that some of these cells were undergoing degenerative changes, the cellular outlines becoming indistinct and the nuclei pycnotic.

There is an extensive literature on the possible localization of secretory function in the various cell types in the pituitary. Most of the evidence concerning the source of the thyrotrophic hormone is derived from studies of the effects of thyroidectomy upon the histology of the pituitary. When the thyroid gland is removed the pituitary responds by an increased production of thyrotrophic hormone and one would assume that the increased activity of the cells which secrete this hormone would be accompanied by some change in their appearance or relative numbers. The results of such investigations have been fully reviewed by Adams (1946) and will not be considered in detail here. While there is some disagreement, most of the work indicates that after thyroidectomy definite changes occur in the basophils. The changes most frequently reported are vacuolization of the cells and an increase in their number. There is considerable divergence of opinion as to whether any significant changes in the acidophils regularly occur.

With the discovery of the goitrogens as tools for carrying out "chemical thyroidectomy," several investigators turned to the use of these drugs as a means

of obtaining further evidence concerning the source of the thyrotrophic hormone. Griesbach (1941) reported that the pituitaries of mammals given a goitrogenic diet exhibit a rapid increase in basophilic elements but that this increase, after reaching a maximum of 56 days, is followed by a return to a more normal condition. The time of return of the pituitary to normal coincided with the time of appearance of colloid in the hyperplastic thyroid glands and Griesbach considered this consistent with the hypothesis that the basophils are the source of the thyrotrophic hormone. Gasche and Druey (1946), who appear to be the only investigators who have previously studied the effect of goitrogens on amphibian pituitaries, refer only briefly to the fact that the pituitaries of *Xenopus laevis* larvae raised in thiourea exhibit the same changes in basophil cells as those which appear after thyroidectomy.

As has been seen, the present work is in agreement with most previous studies in that interference with the normal functioning of the thyroid gland was followed by an increase in the number of basophils in the anterior lobe of the pituitary. The results of long-continued treatment with goitrogens, however, do not agree with those reported by Griesbach. The "regression" which occurred in the thyroid was not accompanied by any return of the pituitary to a normal histological condition; instead, the pituitary showed increasing abnormality with signs of degeneration of some of the basophilic elements. This, however, does not seem surprising since the decreased thyroid activity is assumed to result from failure of the thyrotrophic hormone-producing cells. In fact, since the present results are in such accord with the changes reported by most authors following thyroidectomy, they may be interpreted as adding one more confirmation to the hypothesis that basophils of the anterior lobe of the pituitary are the sources of the thyrotrophic hormone. It must be pointed out, however, that there is another possible interpretation of the pituitary changes. Long-continued treatment with thiourea and similar substances may have direct effects upon the histology of the pituitary and the changes observed therefore may rest upon this basis rather than upon the over-stimulation of thyrotrophic hormone production resulting from thyroid inhibition. So far as the author is aware none of the studies of the results of administration of goitrogens have eliminated the possibility that these drugs might have selective effects upon certain cellular elements in the pituitary which after a time might cause degenerative changes in these elements. It is planned to study the pituitaries of a series of animals given simultaneous treatment with thyroxin and thiourea. Presumably the administration of thyroxin at an appropriate concentration would prevent any stimulation of the pituitary to over-production of thyrotrophic hormone and if any changes in the pituitary then occur such changes could be ascribed to direct effects of the thioura.

Summary

1. A study of the effect of various concentrations of four thyroid-inhibiting substances upon thyroid and pituitary glands in *Desmognathus fuscus brimleyorum* Stejneger has been made.

2. The optimal concentration for thiourea was found to be in the region of 0.01% to 0.3%.

3. The order of effectiveness of the goitrogens tested was phenylthiourea, allylthiourea, thiourea and thiouracil.

4. After long-continued treatment with concentrations of optimal efficiency, the thyroid showed histological evidence of a decrease in activity.

5. Pituitary preparations showed an increase in basophilia as a result of the treatment.

6. Pituitaries of animals which had shown decreased activity in the thyroid preparations exhibited an onset of degeneration in the basophilic elements.

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